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From: Hunt, Jennifer
Sent: Sunday, November 04, 2001 12:29 PM
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Subject: References for 09/218,481

Please send me the following references ASAP:

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Endothelial Cell Dysfunct. (1992), 477-503

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Thanks,

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1265 Cellular Responses to PDGF-AB After Down Regulation of PDGF-α Receptors: Evidence that Functional PDGF Binding Does Not Require Receptor Dimerization. V. Drozdoff and W.J. Pledger, Department of Cell Biology, Vanderbilt University School of Medicine, Nashville, TN 37232.

Platelet derived growth factor (PDGF) and its receptor have been found to exist in multiple forms. PDGF exists in three dimeric combinations of A and B subunit chains, which are the products of separate genes. The PDGF receptor is similarly encoded by genes for two distinct receptor proteins, α and β. A recent model proposed PDGF binding involves the association of the two receptor proteins into three possible dimeric forms. An essential prediction of that model is that PDGF α-receptors are required for cells to bind and respond to the heterodimeric AB isoform of PDGF. In agreement, we found PDGF-AB stimulation of receptor autophosphorylation was dependant on the presence of α-receptors, suggesting receptor dimerization was required to induce autophosphorylation of the β-receptor. However, in contrast, we present evidence that both binding and functional response to PDGF-AB was retained in Balb/c-3T3 cells after PDGF α-receptors had been down regulated by PDGF-AA pretreatment. This suggests initial receptor activation and early PDGF-stimulated events may occur independently of both receptor autophosphorylation and dimerization. We propose that some cellular responses to PDGF isoforms may be mediated by only two functional receptor classes and describe how this model may also account for the binding specificity of the PDGF isoforms.

1266 Purification of a Smooth Muscle Cell Mitogen From Beta Tumor Cell Conditioned Medium. Y. Shing, X. Tao, C. Butterfield, D. Hanahan, and J. Folkman. The Children's Hospital and Harvard Medical School, Boston, MA 02115, and Department of Biochemistry, University of California, San Francisco, CA 94143.

A novel growth factor (BTC-GF) was purified from the conditioned medium of pancreatic beta tumor cells initially derived from transgenic mice (RIP1-Tag2) in which virtually every beta cell expressed the oncogene SV40 large T. The purification scheme included BioRex 70 chromatography, phenyl-Sepharose chromatography, TSK-GEL heparin FPLC and C4 reverse phase HPLC. Purification was guided by DNA synthesis in 3T3 cells. The peptide also stimulated proliferation of bovine smooth muscle cells. It was not inactivated by boiling, by 10mM dithiothreitol or by exposure to 1M acetic acid. The biological activity of BTC-GF was recovered from a single band of protein which had a molecular weight of 32,000 on SDS-PAGE. The partial N-terminal amino acid sequence of this protein was determined with an ABI 470A protein sequencer, and a computer search based on this sequence through translated GENBANK version 63 and NBRF Protein Database version 24 indicates that BTC-GF is a new peptide. (Supported by USPHS Grant #2R37CA37395-09 and by a grant to Harvard University from Takeda Chemical Industries, Ltd.)

1267 Conditioned Medium From A Glioblastoma Cell Line Contains A Protein That Reacts With An Antibody To Vascular Permeability Factor. I. F. Megyesi, R. A. Rosenthal and J. Folkman. Department of Surgery, Children's Hospital and Harvard Medical School, Boston, MA 02115

Human glioblastoma multiforme, a type of brain tumor, is among the most vascularized neoplasms (Brem *et al.*, J Natl Cancer Inst 48:347-356, 1972) . It has been proposed that glioblastoma cells may secrete a factor, or factors, that stimulate the growth of endothelial cells. Glioblastoma cells may also secrete a factor which modifies vascular permeability, since these tumors are often associated with vasogenic cerebral edema. A candidate for such a factor is vascular permeability factor (VPF) (Senger *et al.*, Cancer Res 50:1774-1778, 1990) which is essentially identical to vascular endothelial growth factor (VEGF) (Keck *et al.*, Science 246:1309-1312, 1989). This secreted protein stimulates the growth of endothelial cells and also increases vascular permeability. In order to determine if human glioblastoma cells secrete VPF, a human glioblastoma cell line was grown in serum free medium. The medium conditioned by these cells stimulated proliferation and DNA synthesis in bovine capillary endothelial cells. A growth factor from the conditioned medium was then partially purified by cation exchange chromatography. The active fractions were subjected to Western blotting using a goat antibody which had been raised against VPF. Immunoreactive bands were detected at 23 kDa and 46 kDa, which correspond to the molecular weights of the monomeric and dimeric forms of VPF respectively. These findings suggest that this human glioblastoma cell line produces VPF. VPF may be at least partially responsible for the increased vascularization and vascular permeability seen in certain glioblastoma tumors.

1268 Isoform Specific Regulation of Platelet-derived Growth Factor (PDGF) Binding and Receptor Turnover in Osteoblast-enriched Cultures from Fetal Rat Bone. M.Centrella, T.L.McCarthy, E.Canalis Research Laboratory and Department of Medicine, Saint Francis Hospital & Medical Center, Hartford, and the Univ. of Connecticut Health Center, Farmington, CT

PDGF exists as a homodimer or a heterodimer comprising PDGF-A or PDGF-B subunits, and their biochemical effects are in part distinguished by differential binding patterns between two binding sites, designated as PDGF-α or PDGF-β receptor subunits. The relative efficacy of the three PDGF isoforms (PDGF-AA, PDGF-AB, and PDGF-BB) on replication and protein synthesis in osteoblast-enriched cultures is graded with regard to PDGF-B subunit content (PDGF-BB being most potent) and β receptor subunit occupancy. We have determined that the modest mitotic response by osteoblasts to PDGF-AA is enhanced by interleukin 1 (IL-1), to essentially the level induced by PDGF-BB; this result is related to a specific increase in effective PDGF-AA binding site number, with no significant changes in PDGF-BB binding observed. Steady state PDGF-AA and PDGF-BB binding levels remain relatively constant in untreated cultures, but cycloheximide treatment, to inhibit de novo protein synthesis, reduces PDGF-AA binding by about 40% within 4 hours, and its binding continues to decline through 24 hours, without affecting PDGF-BB binding. Also, the stimulatory effect of IL-1 on PDGF-AA binding is nearly completely blocked by cycloheximide. These studies indicate that PDGF-AA, but not PDGF-BB, binding sites are rapidly turned over in osteoblast-enriched cultures, and that the stimulatory effect of IL-1 in this model is regulated, at least in part, by increasing new PDGF-α receptor synthesis.

1269 S100β stimulates glial cell proliferation. S.W. Barger, R.H. Selinfreund, and L.J. Van Eldik, Departments of Pharmacology and Cell Biology, Vanderbilt University, Nashville TN 37232.

S100β is a calcium binding protein found in abundance in glial cells of the brain. S100β is released from glial cells, and a disulfide-linked form of the protein has neurotrophic activity on selected neuronal cells (e.g., cortical, spinal cord, and dorsal root ganglia neurons). We report here that, in addition to its neurotrophic activity on neurons, S100β has growth factor activity on glial cells. Initial studies (Selinfreund et al., J Cell Biol., in press) showed that selective decreases in S100β levels in rat C6 glioma cells by use of antisense oligodeoxynucleotides correlated with a decrease in cellular growth rate. More recently, we have found that addition of S100β to growth-arrested, subconfluent cultures of rat primary astrocytes or C6 glioma cells stimulated cell proliferation in a dose-dependent manner. Treatment of cells with S100β also resulted in increases in 3H-thymidine incorporation and changes in protooncogene mRNA levels. Under similar conditions, S100β was unable to stimulate proliferation of two neuroblastoma cell lines. These data suggest that S100β may play regulatory roles both in glial cell proliferation and in neuronal differentiation in the central nervous system. (Supported in part by funds from Muscular Dystrophy Association and Cystic Fibrosis Foundation).

1270 Expression of c-fos and c-myc in Human Fibroblasts. N. Bi and M.D. Mumrack, Wright State University, Dayton, Ohio 45435.

WS-1 cells are a human diploid fibroblast strain derived from embryonic skin. WS-1 cells quiesce during 48 hr in serum-free medium and undergo DNA synthesis when stimulated by 10% fetal bovine serum or 10 nM thrombin. Thrombin rapidly stimulates the production of inositol phosphates, a transient increase in intracellular calcium, and a rise in intracellular pH within 1-2 minutes. Phorbol myristate acetate (PMA) does not stimulate DNA synthesis, production of inositol phosphates, or an increase in intracellular calcium, but PMA does increase intracellular pH. In quiescent WS-1 cells, thrombin or PMA causes an increase in c-fos and c-myc gene transcripts. Maximal levels of c-fos and c-myc transcripts accumulate within 15-45 minutes following stimulation. The c-fos mRNA levels return to control values in 2 hr. Staurosporine (50nM), a protein kinase C inhibitor, inhibits the increase in c-myc gene expression stimulated by thrombin, serum, or PMA. Staurosporine inhibits the increase in c-fos stimulated by PMA but not thrombin and serum. The lack of inhibition by staurosporine suggests that thrombin- and serum-induced c-fos gene expression may use pathways other than protein kinase C. (Supported in part by the American Heart Association, Ohio Affiliate)